Claims

1. A method for producing at least one proteinaceous substance in a eukaryotic cell, said method comprising:

providing a eukaryotic cell having a nucleic acid sequence in the eukaryotic cell's genome, said nucleic acid sequence encoding at least one adenoviral E1 protein or a functional homologue, fragment or derivative thereof, which eukaryotic cell further does not encode a structural adenoviral protein in its genome or a sequence integrated therein;

providing said eukaryotic cell with a gene encoding a recombinant proteinaceous substance;

culturing said eukaryotic cell in a suitable medium; and

harvesting at least one proteinaceous substance from said eukaryotic cell, said suitable medium, or both said eukaryotic cell and said medium.

2. A method for enhancing production of a recombinant proteinaceous substance in a eukaryotic cell, said method comprising:

providing said eukaryotic cell with a gene encoding at least part of a proteinaceous substance, wherein said nucleic acid is under control of a CMV-promoter, an E1A promoter, or a functional homologue, derivative and/or fragment of either; and

providing said eukaryotic cell with adenoviral E1A-activity or E1A-like activity.

3. The method according to claim 1 or claim 2, wherein said eukaryotic cell is a mammalian cell.

4. The method according to claim 3, wherein said eukaryotic cell is a human cell.

5. The method according to any one of claims 1 to 4, wherein at least one of the at least one proteinaceous substance harvested is encoded by said gene.

6. A method for producing at least one human recombinant protein in a cell, said method comprising:

providing a eukaryotic cell which is human, with a gene encoding a human recombinant protein, having a sequence encoding at least one adenoviral E1 protein or a functional derivative, homologue or fragment thereof in the human cell's genome which human cell further does not produce structural adenoviral proteins;

culturing said human cell in a suitable medium; and

harvesting the human recombinant protein from the human cell, the suitable medium, or both said human cell and said medium.

7. The method according to any one of the aforegoing claims, wherein said at least one adenoviral E1 protein comprises an E1A protein or a functional homologue, fragment and/or derivative thereof.

8. The method according to any one of the aforegoing claims, wherein said at least one adenoviral E1 protein comprises an E1B protein or a functional homologue, fragment and/or derivative thereof.

9. The method according to any one of claims 1 through 8, wherein said eukaryotic cell produces from about 2 to about 200-fold more recombinant protein and/or proteinaceous substance than conventional mammalian cell lines.

10. The method according to claim 9, wherein said conventional mammalian cell lines are selected from the group consisting of CHO, COS, Vero, Hela, BHK and Sp-2 cell lines.

11. The method according to any one of claims 1 to 10 wherein said proteinaceous substance is a protein that undergoes post-translational and/or peri-translational modifications.

12. The method according to claim 11, wherein said post-translational and/or peri-translational modifications comprise glycosylation.

13. The method according to any one of claims 1-12, wherein said proteinaceous substance is erythropoietin, or a functional derivative, homologue or fragment thereof.

14. The method according to claim 13, wherein said eukaryotic cell produces in excess of 100 units erythropoietin thereof per million cells in 24 hours.

15. The method according to claim 14, wherein said eukaryotic cell produces in excess of 500 units erythropoietin thereof per million cells in 24 hours.

16. The method according to claim 15, wherein said eukaryotic cell produces in excess of 1000 units erythropoietin thereof per million cells in 24 hours.

17. The method according to claim 16, wherein said eukaryotic cell produces in excess of 5000 units erythropoietin or functional derivatives thereof per million cells in 24 hours.

18. A recombinant mammalian cell immortalized by the presence of at least one adenoviral E1 protein or a functional derivative, homologue and/or fragment thereof, said recombinant mammalian cell comprising a nucleic acid in a functional format for expressing at least one variable domain of an immunoglobulin or a functional derivative, homologue and/or fragment thereof.

19. The recombinant mammalian cell of claim 18, wherein said at least one adenoviral E1 protein comprises an E1A protein or a functional homologue, fragment and/or derivative thereof.

20. The recombinant mammalian cell of claim 18 or claim 19, wherein said at least one adenoviral E1 protein comprises an E1B protein or a functional homologue, fragment and/or derivative thereof.

21. The recombinant mammalian cell of any one of claims 18 to 20, comprising a nucleic acid derived from an adenovirus encoding said at least one adenoviral E1 protein.

22. The recombinant mammalian cell of claim 21, wherein said nucleic acid derived from an adenovirus encodes an E1A and/or an E1B protein.

23. The recombinant mammalian cell of any one of claims 18-22, wherein said recombinant mammalian cell is derived from a primary cell.

24. The recombinant mammalian cell of any one of claims 18 through 23, which recombinant mammalian cell is derived from a human cell.

25. The recombinant mammalian cell according to claim 24, deposited as ECACC no. 96022940 or a derivative thereof.

26. The recombinant mammalian cell of any one of claims 18 through 25, wherein said cell further comprises a nucleic acid encoding E2A or a functional homologue, fragment and/or derivative thereof.

27. The recombinant mammalian cell of claim 26, wherein said nucleic acid encoding E2A comprises a temperature sensitive mutant E2A.

28. The recombinant mammalian cell of any one of claims 18 through 27, wherein said nucleic acid in a functional format for expressing at least one variable domain, encodes a heavy chain, a variable heavy chain, a light chain and/or a variable light chain of an immunoglobulin.

29. The recombinant mammalian cell of any one of claims 18 through 28, further comprising another nucleic acid in functional format for expressing at least one counterpart of said at least one variable domain.

30. The recombinant mammalian cell of any one of claims 18 through 29, wherein said nucleic acid in functional format for expressing at least one variable domain and/or at least one counterpart thereof encodes an ScFv.

31. The recombinant mammalian cell of any one of claims 18 through 30, wherein at least one of said variable domains comprises a human or humanized amino acid sequence.

32. The recombinant mammalian cell of any one of claim 18 through 31, wherein at least one of said variable domains is encoded by a nucleic acid under the control of an inducible promoter.

33. A process for producing at least one variable domain of an immunoglobulin, said process comprising:

culturing a recombinant mammalian cell of any one of claims 18-32, in a suitable medium; and

harvesting said at least one variable domain of an immunoglobulin from said recombinant mammalian cell and/or said medium.

34. The process according to claim 33, wherein said recombinant mammalian cell is capable of producing in excess of 10 grams of said at least one variable domain of an immunoglobulin per 106 cells per day.

35. A process for producing at least one variable domain of an immunoglobulin having post-translational modifications different than that of the variable domain of an immunoglobulin's isolated natural counterparts, said process comprising:

transforming the recombinant mammalian cell of any one of claims 18 through 32 with a gene coding for the variable domain of the immunoglobulin;

culturing the recombinant mammalian cell in a suitable medium; and

harvesting the at least one variable domain of an immunoglobulin from said recombinant mammalian cell and/or said suitable medium.

36. The process according to claim 35, wherein said recombinant mammalian cell produces said at least one variable domain of an immunoglobulin, in excess of 10 g per 106 cells per day.

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37. A variable domain of an immunoglobulin, or a functional part, homologue or derivative thereof, produced by the process of any one of claims 33 through 36.

38. The variable domain of an immunoglobulin according to claim 37 together with a suitable carrier forming a pharmaceutical composition.

39. The method according to any one of claims 1-12, wherein said proteinaceous substance comprises a viral protein other than an adenoviral protein.

40. The method according to claim 39, wherein said viral protein comprises an influenza virus neuramidase and/or a hemagglutinin.

41. The method according to claim 39, wherein said viral protein comprises an enterovirus protein or a functional equivalent thereof

42. The method according to claim 41, wherein said enterovirus protein is selected from the group consisting of rhinovirus, aphto virus, and poliomyelitis virus protein.

43. The method according to claim 39, wherein said viral protein comprises a herpes virus protein or a functional equivalent thereof.

44. The method according to claim 43, wherein said herpes virus protein comprises a protein selected from the group consisting of herpes simplex virus, pseudorabies virus and bovine herpes virus protein.

45. The method according to claim 39, wherein said virus protein comprises an orthomyxovirus protein.

46. The method according to claim 45, wherein said orthomyxovirus protein is selected from the group consisting of an influenza virus, a paramyxovirus, such as Newcastle Disease virus, a respiratory syncitio virus, a mumps virus and a measles virus protein.

47. The method according to claim 39, wherein said virus protein comprises a retrovirus, a parvovirus or a popavovirus protein.

48. The method according to claim 47, wherein said retrovirus protein comprises a human immunodeficiency virus protein.

49. The method according to claim 39, wherein said virus protein comprises a rotavirus or a coronavirus protein.

50. The method according to claim 49, wherein said rotavirus or coronavirus protein is selected from the group consisting of a transmissible gastroenteritisvirus or a flavivirus, such as tick-borne encephalitis virus and yellow fever virus protein.

51. The method according to claim 39, wherein said virus protein comprises a togavirus protein, rubella virus protein or an Eastern-, Western-, or Venezuelan equine encephalomyelitis virus protein.

52. The method according to claim 39, wherein said virus protein comprises a hepatitis causing virus protein, a hepatitis A protein, or a hepatitis B virus protein.

53. The method according to claim 39, wherein said virus protein comprises a pestivirus protein, such as hog cholera virus protein or a rhabdovirus protein, such as a rabies virus protein.

54. A process for producing a vaccine comprising a viral protein, said process comprising:

producing the viral protein in a human cell having a sequence encoding at least one adenoviral E1 protein or a functional derivative, homologue or fragment thereof in the human cell's genome, which human cell does not produce a structural adenoviral protein;

harvesting the viral protein;

incorporating the thus harvested viral protein in a vaccine.

55. The method according to any one of claims 1-17, 33, 34, or 39-53, wherein said eukaryotic cell is derived from a primary cell.

56. The method according to any one of claims 1-17, 33, 34, 39-53 or 55, wherein said eukaryotic cell is immortalized by the presence of said E1 encoding sequence.

57. The method according to any one of claims 1-17, 33, 34, 39-53, 55 or 56, wherein said eukaryotic cell further comprises a sequence encoding E2A or a functional derivative or analogue or fragment thereof in its genome.

58. The method according to claim 57, wherein said E2A encoding sequence encodes a temperature sensitive mutant E2A.

59. The method according to any of claims any one of claims 1-17, 33, 34, 39-53, 55-58, wherein said human cell comprises no other adenoviral sequences.

60. The method according to any one of claims 1-17, 33, 34, 39-53, 55-59, wherein said human cell grows in suspension.

61. The method according to any one of claims 1-17, 33, 34, 39-53, 55-60, wherein said eukaryotic cell is the PER.C6 cell as deposited under ECACC no. 96022940 or a derivative thereof.

62. The method according to any one of claims 1-17, 33, 34, 39-53, 55-61, wherein said human cell is cultured in the absence of serum.

Sub A12

63. A recombinant erythropoietin molecule obtainable by a method according to any one of claims 1-17 and 55-62.

64. The recombinant protein claim 63 wherein said recombinant protein has a human glycosylation pattern different from that of the protein's isolated natural counterpart protein.

65. A human cell having a sequence encoding at least one E1 protein of an adenovirus or a functional derivative, homologue or fragment thereof in its genome, which cell does not produce structural adenoviral proteins and having a gene encoding a recombinant protein.

66. The human cell of claim 65 which is derived from PER.C6 as deposited under ECACC no. 96022940

67. The human cell of claim 65 or 66, which further comprises a sequence encoding E2A or a functional derivative or analogue or fragment thereof in its genome.

68. The human cell of claim 67, wherein said E2A is temperature sensitive.

69. A method of enhancing the production of a proteinaceous substance in a eukaryotic cell an adenoviral E1B protein or a functional derivative, homologue and/or fragment thereof having anti-apoptotic activity, said method comprising providing said eukaryotic cell with an adenoviral E1B protein, derivative, homologue and/or fragment thereof.

70. The process according to claim 54, wherein said human cell further comprises a sequence encoding E2A or a functional derivative or analogue or fragment thereof in the human cell's genome.

71. The process according to claim 70, wherein said E2A encoding sequence encodes a temperature sensitive mutant E2A.

Sub A13 72. The recombinant mammalian cell of any one of claim 18 through 31, wherein an endogenous DHFR nucleic acid is at least functionally deleted.